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Award Number: DAMD17-01-1-0359

TITLE: Novel Strategies for the Identification and Characterization of Selective Estrogen Receptor Modulators (SERMs)

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REPORT DATE: October 2002

TYPE OF REPORT: Annual

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 074-0188

1. AGENCY USE ONLY (Leave blank)	2. REPORT DATE	3. REPORT TYPE AND DATES COVERED
	October 2002	Annual (24 Sep 01 - 23 Sep 02)

4. TITLE AND SUBTITLE
Novel Strategies for the Identification and Characterization of Selective Estrogen Receptor Modulators (SERMs)

5. FUNDING NUMBERS
DAMD17-01-1-0359

6. AUTHOR(S):
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8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)
U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

10. SPONSORING / MONITORING AGENCY REPORT NUMBER

11. SUPPLEMENTARY NOTES

12a. DISTRIBUTION / AVAILABILITY STATEMENT
Approved for Public Release; Distribution Unlimited

12b. DISTRIBUTION CODE

13. Abstract *(Maximum 200 Words)* *(abstract should contain no proprietary or confidential information)*

Estrogens promote growth of certain breast and endometrial cancers. Selective estrogen receptor modulators (SERMs), ligands that block the activity of estrogen in only selective tissues, allow to control the growth of these cancers while avoiding the many unwanted side effects associated with the use of antiestrogens. The goal of our work is to identify the mechanisms that control the activity of SERMs and to develop efficient high-throughput strategies for their identification.

SERMs exert their activities by binding to the two estrogen receptors, ERα and ERβ. Ligand binding changes the position of α–helix 12 (H12) in the ER ligand-binding domain and regulates the interaction of ERs with cellular cofactors. We identified that the F-domain, a receptor-specific domain that follows H12, contributes to coactivator binding and specificity of ERα but not ERβ. Thus, this domain appears to be involved in the differential response of ERα and ERβ to particular SERMs, likely by modulating the ligand-induced repositioning of H12. To monitor the dynamics and structural reorganization of ERα and ERβ upon SERM binding, we are exploring experimental strategies to introduce fluorescent labels into H12 or the F-domain. These fluorescent-labeled receptors will also allow us to identify novel SERMs in high throughput screens.

14. SUBJECT TERMS	15. NUMBER OF PAGES
breast cancer, estrogen receptor, selective estrogen receptor modulators (SERMs), structure/function, drug design	13
	16. PRICE CODE

17. SECURITY CLASSIFICATION OF REPORT	18. SECURITY CLASSIFICATION OF THIS PAGE	19. SECURITY CLASSIFICATION OF ABSTRACT	20. LIMITATION OF ABSTRACT
Unclassified	Unclassified	Unclassified	Unlimited

Table of Contents

Cover	1
SF 298	2
Introduction	4
Body	4
Key Research Accomplishments	9
Reportable Outcomes	10
Conclusions	10
References	10
Appendices	11

Introduction

Estrogens promote the growth of particular tissues, and are involved in the cause of breast and endometrial cancers. Antiestrogens block the activity of estrogens and play important roles in the treatment of these cancers. However, they have many unwanted side effects such as increased risk for osteoporosis and heart diseases. Selective estrogen receptor modulators (SERMs) provide a powerful alternative to antiestrogens. SERMs display profound cell-specific activity profiles blocking the activity of estrogens in selective tissues such as breast. The goal of our work is to identify the mechanisms that control the activity of SERMs and to develop efficient high-throughput strategies for their identification.

Like natural estrogens, SERMs exert their activities by binding to the two estrogen receptors, ERα and ERβ, which are ligand-regulated transcription factors. Upon ligand binding, the position of α–helix 12 (H12) in the ligand-binding domain of ERα and ERβ changes in a ligand-specific manner and regulates the interaction of these receptors with cellular corepressors and coactivators. The current hypothesis is that the cell-specific activity of SERMs is caused by cellular differences in the repertoir of coactivators and corepressors that recognize particular, SERM-induced receptor conformations.

During the past year our work has been focused on the identification of receptor-specific structural elements involved in the ligand-dependent repositioning of H12 and in the recruitment of corepressors and coactivators. We identified that the F-domain, a receptor-specific domain that follows H12, differentially enables ERα and ERβ to distinguish between different coactivators. Thus, this domain appears to be involved in the differential response of ERα and ERβ to particular SERMs, likely by modulating the ligand-induced repositioning of H12. We have been exploring experimental strategies to introduce fluorescent labels into H12 or the F-domain. These fluorescent-labeled receptors will allow us to monitor the dynamics and structural reorganization of ERα and ERβ upon SERM binding or heterodimerization and constitute the first step in the development of a high-throughput screen to identify novel SERMs.

Body

Our revised "STATEMENT OF WORK" (08/10/01) contains three specific aims:

1. Monitor the location and dynamic of α-helix 12 of ER in the absence and presence of various estrogens and develop a high throughput screen for the identification of new potential SERMs
2. Analyze the role of the ER F-domain in the ligand-dependent relocation of α-helix 12
3. Probe for α-helix 12-dependent structural changes in ERα and ERβ homo- and heterodimers

During the past year we focused on aim 1 and 2. Since the results obtained in aim 2 are important for aim 1, I will begin to outline our accomplishments for aim 2.

Aim 2. Analyze the role of the ER F-domain in the ligand-dependent relocation of α-helix 12

In addition to very different N-terminal domains, ERα and ERβ are characterized by very diverse F-domains. These domains follow helix 12 in the ligand binding domain and play important roles in the ligand interpretation of steroid receptors. Mutations in the F-domain have been identified that enable steroid receptors to activate transcription in the presence of antagonists [Montano et al., 1996; Nichols et al., 1998]. The recently solved structures of the progesterone, and glucocorticoid receptors demonstrated that in the presence of agonists the F-domain is linked to the ligand binding domain via a β-strand [Williams and Sigler, 1998; Bledsoe et al., 2002]. This suggests that the F-domain might restrict the mobility of H12 and modulate the ligand-induced relocation of this helix. Moreover, since the the F-domain is located close to the coactivator interaction site it is likely that the F-domain influences coactivator binding and selectivity of the ER LBD. Thus, the F-domain appears to be an important factor in the ligand-dependent activation of ERs. However, since the ER F-domain seems to impede crystallization, all available ER structures miss this important domain. The goal of the following experiments is to evaluate the role of the F-domain for the ability of ER to interact with ligands and coactivators and to investigate the influence of the F-domain on the ligand-dependent relocation of H12.

Aim 2a: Characterization of ERα/β±F domain

Cloning, expression and purification of human ERα/β LBD±F domain - Following the outline presented in the revised "STATEMENT OF WORK" (08/10/01) aim 2a, we have cloned the ligand binding domains of human ERα and ERβ in the absence and presence of the F-domain using a PCR strategy with human brain cDNA as template. These PCR products were cloned into bacterial and mammalian expression vectors and verified by sequence analysis. Expression in the *E. coli* strain BL21DE3 revealed that ERβ and ERβ-F are at least partially soluble, whereas ERα and ERα-F were completely insoluble. However, decreasing the temperature during the expression to 13°C and coexpression of the chaperones GROEL/ES increased the solubility and enabled us to obtain between 2 and 8 mg soluble protein per liter bacterial culture for all four ER constructs. With the help of a His_6-tag added to the N-terminus of these ER ligand binding domains, all ER LBDs (ERα, ERα-F, ERβ, ERβ-F) were purified close to homogeneity using a Talon-affinity column.

Hormone binding of ERα/β LBD±F domain - These purified proteins bind 17-β estradiol with the following dissociation constants: ERα/1.0±0.5 nM, ERα-F/0.5±0.2 nM, ERβ/1.4±0.5 nM, and ERβ-F/1.2±0.3 nM. Thus, in case of ERβ the F-domain does not change estradiol binding, whereas removal of the ERα F-domain increases the affinity for estradiol by a factor 2. Binding studies with 4 OH-tamoxifen are in progress.

Coactivator binding of ERα/β LBD±F domain - The transcriptional activity of ER depends on its interaction with coactivators such as the p160 coactivator GRIP1. Most coactivators have multiple nuclear receptor interaction sites, called NR-boxes, which differ in their affinity for different receptors. Using quantitative GST-pulldown assays [Darimont et al., 1998] we determined that ERα displays a higher affinity for the GRIP1 NR-boxes 1 and 2 than for NR-box 3, whereas ERβ does not discriminate between these NR-boxes (Fig. 1). Deletion of the F-domain increases binding of ERα to the nuclear receptor interaction domain of GRIP1 while abolishing the selectivity for particular GRIP1 NR-boxes. Deletion of ERβ F-domain has no obvious consequence for the binding of GRIP1 (Fig. 1). These results demonstrate that the F-domain of ERα, but not that of ERβ, is important for coactivator binding. Thus, the F-domain likely contributes to the observed differences in the hormone responsiveness of ERα and ERβ.

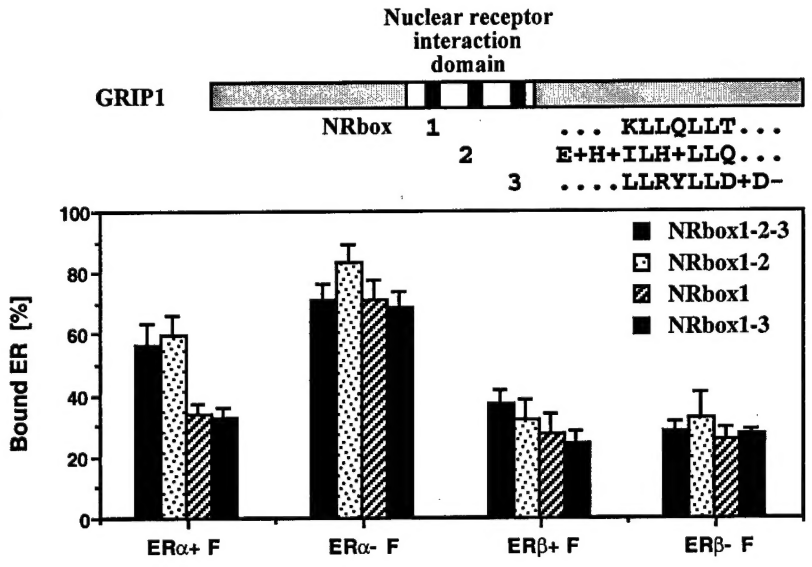

Fig. 1 Coactivator binding of ERs

The p160 coactivator GRIP1 contains three NRboxes with the characteristic "LxxLL" nuclear receptor-binding motif. To determine whether the F-domain contributes to coactivator binding and selectivity, we measured binding of ^{35}S-labeled ERα/β LBD in the absence and presence of the F-domain to glutathione-S-transferase fusions of GRIP1 nuclear receptor interaction domain variants that contain all three NR-boxes, NRbox 1 and 2, NRbox 1, or NRbox 1 and 3. NR-boxes were mutated by replacing the conserved LxxLL (L=leucine) motifs with AxxAA (A=alanine). The amount of bound receptor (relative to input) was quantified using a phosphorimager. The shown results are the average of more than three independent experiments.

Transcriptional activity of ERα/β ±F domain - To determine whether the observed changes in coactivator binding leads to changes in the transcriptional activity of the ERs, we investigated the transcriptional activity of ERα and ERβ in the absence and presence of the F-domain in transiently transfected CV1 cells using a reporter-based activity assay. In agreement with the coactivator binding studies, the presence or absence of the F-domain did not change

the transcriptional activity of ERβ, whereas in the absence of the F-domain the efficacy of the transcriptional activity of ERα increased 2-fold.

Aim 2b: Monitoring the ligand-dependent localization of the F-domain

A possible explanation for the differences in the roles of the ERα and ERβ F-domains might be that the F-domain of ERα is linked back to the ER LBD core and controls the ligand-dependent localization of H12, whereas the F-domain of ERβ is a flexible, solvent exposed extension of H12, which does not influence the movement of H12. Upon labeling the F-domains with a fluorophore, these structural differences could be monitored using time resolved fluorescence anisotropy.

Selection of a labeling system - To identify an efficient labeling strategy and to test whether structural changes in the F-domain could be detected by this strategy, in a pilot study we used the progesterone receptor (PR), for which the structure and localization of the F-domain has been determined by X-ray crystallography [Williams and Sigler, 1998]. We decided to attempt labeling of this receptor using a protein splicing approach as outlined in Fig. 2A. In this system (developed by New England Biolabs), a cysteine residue is introduced at the C-terminal end of H12 in the PR LBD, which is fused to the yeast protein intein bound to an affinity matrix. Intein mediates protein splicing by catalyzing transesteration reactions between C-terminal and N-terminal cysteine residues. The F-domain, containing an N-terminal cysteine residue, is synthesized and fluorescently labeled *in vitro*. This peptide is then ligated to the PR LBD through an intein-catalyzed transesteration reaction between the cysteine residue at the C-terminal end of H12 and the N-terminal cysteine in the fluorescent-labeled F-domain. This reaction also releases the labeled PR LBD+F-domain from the affinity matrix. A final gelfiltration step separates the labeled PR LBD+F-domain from the free fluorescent-labeled F-domain peptide. Time-dependent fluorescence anisotropy measurements in the absence or presence of agonists and antagonists will allow us to monitor ligand-dependent changes in the mobility of the F-domain. With respect to the known crystal PR structure, in the presence of agonists we expect only a slow rotational movement of the fluorophore which corresponds to the rotation of the entire protein.

Construction, expression and purification of the PR LBD-Intein fusion protein - Based on the available structural information [Williams and Sigler, 1998] we decided to introduce the C-terminal cysteine residue at position A922 in the PR LBD and the fluorophore at position P927 (Fig. 2B). P927 follows the short β-strand in the PR LBD, which in the presence of agonists links the PR F-domain back to the LBD. Thus, the rotational freedom of fluorophores introduced at this position should be constrained upon the formation of the β-sheet that links the F-domain to the LBD core. The PR LBD-Intein was constructed using a PCR strategy and verified by DNA sequencing. Upon expression in *E. coli*, at room temperature about 90% of the expressed protein was found to be insoluble. The remaining 10% (about 0.5 mg/ liter culture) bound to chitin with high affinity. This simple affinity chromatography step yielded protein that is more than 90% pure. Incubation of 2 mg chitin-bound PR LBD-Intein with a 10-fold excess of F-domain peptide (synthesized by our Biotech core facility) resulted in 200 µg more than 95% pure PR LBD-F-domain protein. Although our experimental strategy definitely needs to be refined, these results are very encouraging.

At present, we are attempting to improve the solubility of the PR LBD-Intein fusion protein by lowering the cultivation temperature and to increase the peptide coupling efficiency by adjusting the relative concentrations of the reaction partners and the buffer conditions. With help from Molecular probes (Eugene, OR) we are in the process of selecting a fluorophore for the labeling of the F-domain and are designing an experimental strategy that will allow us to introduce this fluorophore during or after peptide synthesis at particular residues. One possible strategy is to replace residue P927 by cysteine and to couple the fluorophore while the N-terminal cysteine is still protected. To investigate whether the replacement of A922 by cysteine will change hormone and coactivator binding of the PR LBD, we are introducing this replacement by site directed mutagenesis and are planning to characterize the mutant protein as outlined in aim 2a. Our goal is to conclude these pilot analyses within the next three months and to proceed with a corresponding analysis of the ligand-dependent localization of the F-domain of ERα and ERβ.

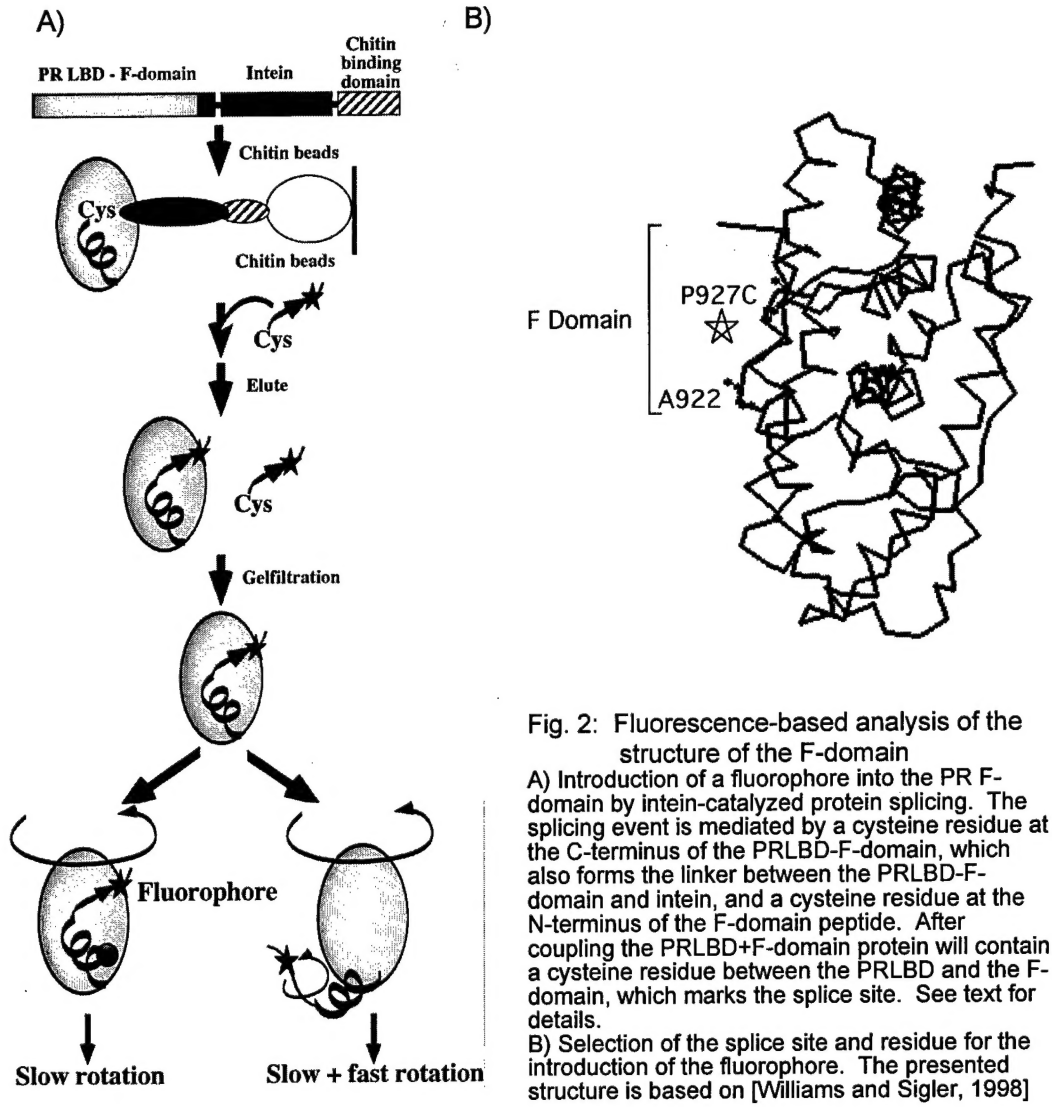

Fig. 2: Fluorescence-based analysis of the structure of the F-domain
A) Introduction of a fluorophore into the PR F-domain by intein-catalyzed protein splicing. The splicing event is mediated by a cysteine residue at the C-terminus of the PRLBD-F-domain, which also forms the linker between the PRLBD-F-domain and intein, and a cysteine residue at the N-terminus of the F-domain peptide. After coupling the PRLBD+F-domain protein will contain a cysteine residue between the PRLBD and the F-domain, which marks the splice site. See text for details.
B) Selection of the splice site and residue for the introduction of the fluorophore. The presented structure is based on [Williams and Sigler, 1998]

Aim 1. Monitor the location and dynamic of α-helix 12 of ER in the absence and presence of various estrogens and develop a high throughput screen for the identification of new potential SERMs

The biological activity of an ER ligand is determined by the cofactors that are recruited by the ligand-bound receptor. Since the recruitment of these cofactors is regulated by the structural changes in the ER induced by the ligand, monitoring these structural changes by fluorescence anisotropy, fluorescence resonance energy transfer or pyrene excimer fluorescence are powerful strategies to characterize ligands and to identify ligands with new activity profiles.

Our results in aim 2 demonstrate that ERα and ERβ have different biochemical features and mechanisms to regulate the recruitment of coactivators. Although it will be ultimately very interesting to compare the structural changes in ERα and ERβ upon binding to particular ligands, to establish the system we decided to focus on ERβ, mainly because this receptor is more soluble. Moreover, since the F-domain of ERβ seems not to contribute to ligand- and coactivator-binding, this receptor allows us to use a C-terminally truncated form of the ERβ LBD for which structural information is available. Thus far, we have performed the majority of the experiments outlined in the revised "STATEMENT OF WORK" (08/10/01) aim 1a and 1b, and we have begun to work out the details of the *in vitro* labeling system that will allow us to introduce fluorophores at specific sites in the ER LBD (aim 1c-e).

Aim 1a: Selection and characterization of sites for the introduction of fluorophores

Criteria for the selection of sites - Ideally, incorporated fluorophores should be solvent-accessable and should not interfere with protein folding, stability, and ligand binding. Moreover donors and acceptors should be located such that the fluorescent properties of donors and acceptors are sensitive to the structural changes induced by the ligand. Based on available structural information [Shiau et al., 1998], we selected the following sites for the introduction of the fluorophores: Potential donor sites - N496, A497, H498; potential acceptor sites - L477 (Agonist monitor), T323 (Antagonist monitor).

Mutational analysis of potential donor sites - To determine whether replacement of the selected sites by a bulky, hydrophobic residue would interfere with protein folding, stability, and ligand binding, we replaced the selected donor sites by tryptophan using site-directed mutagenesis, expressed the mutant proteins in context of ERβ±F-domain in *E. coli*, purified the protein and measured the affinity of these proteins for estradiol. In the presence of the F-domain the mutants N496W, A497W and H498W bind estradiol with similar affinity than wild type ER (1.2±0.3 nM). However in the absence of the F-domain the affinity of N496W and A497W decreased by a factor two, whereas the affinity of H498 for estradiol remained unchanged. Thus, H498 seems to be the best candidate residue for the introduction of the fluorescence donor.

We are in the process of performing similar studies with the selected acceptor sites.

Aim 1b: Analysis of *in vitro* protein expression systems for the introduction of fluorophores by nonsense-codon suppression

As outlined in the original proposal, we plan to incorporate the fluorophores into the ER LBD using a nonsense-codon suppression strategy. This strategy requires a very efficient protein *in vitro* translation system. To identify the optimal system for our purpose, we compared the overall yield and hormone-binding activity of ERβ LBD expressed i*n vitro* using bacterial, wheat germ or rabbit reticulocyte lysates. We found that the overall protein yield was highest in the bacterial expression system, however the yield of hormone-binding competent receptor was significantly higher for proteins expressed in reticulocyte lysate (*E.coli* - yield: 10-50 µg protein/ ml lysate, 50-60% hormone binding competent; rabbit reticulocyte lysate - yield 0.5-5 µg protein/ ml lysate, up to 90% hormone binding competent). We attribute the increased hormone binding competence of the proteins expressed in reticulocyte lysate to the presence of the Hsp90 chaperone complex, which is known to stabilize the hormone-binding-competent conformation of steroid receptors. Unless we find an efficient strategy to separate apo- and holo-receptors, the rabbit reticulocyte systems appears to be the most effective ER *in vitro* translation system. Currently, we are comparing the activities of rabbit reticulocyte lysates from various vendors.

Aim 1c-e: Introduction of fluorescence labels in the ER LBD

Introduction of fluorophores by nonsense-codon suppression is a difficult experimental strategy that requires careful planning of every experimental step. With the help of Dr. Peggy Saks, an expert in tRNA evolution and protein synthesis [Saks et al., 1996], we have begun to select the features of the tRNA that will be used to introduce the labeled residue, and to develop a strategy to prevent that this tRNA is recognized by aminoacyl synthetases present in the lysate. With the help of Molecular Probes (Eugene, OR) we are surveying fluorescence donor and acceptor pairs with respect to their fluorescence properties and their likely tolerance by the ribosome.

While these studies are in progress, we decided to introduce a fluorophore into position 498 in H12 of the ERβ using an intein-catalyzed protein labeling strategy as outlined in aim 2b. Monitoring the rotational freedom of H12 in the presence of various ligands by time resolved fluorescence anisotropy will not only allow us to gain information about the dynamics of H12, but will also enable us to determine whether pyrene excimer fluorescence or fluorescence resonance energy transfer should be used to monitor ligand-dependent structural changes in the ER LBD.

Key Research Accomplishments

Aim 2. Analyze the role of the ER Fdomain in the ligand-dependent relocation of H12

ad Aim 2a:

- Construction, cloning, expression and purification of the ligand binding domains of human ERα and ERβ ± F-domains: The four ER variants can be expressed in *E. coli*, although in the case of ERα low temperature and coexpression of chaperones are necessary in order to obtain soluble protein.
- Estradiol-binding of ERα and ERβ ± F-domains expressed in bacterial and mammalian expression systems: The ERβ F-domain has no influence on estradiol binding, whereas in the absence of the F-domain estradiol binding of ERα is slightly increased.
- Coactivator-binding of ERα and ERβ ± F-domains: The F-domain of ERβ does not modulate binding of the coactivator GRIP1, whereas in the absence of the F-domain the affinity of ERα for GRIP1 is increased and the selectivity of ERα for particular NR-boxes (ER interaction motifs) is lost.
- In agreement with the coactivator interaction studies in reporter-based activity assays using transiently transfected CV1 cells the transcriptional activity of ERα increased about 2-fold in the absence of the F-domain.

These results demonstrate that the F-domains of ERα and ERβ differ in their contribution to ligand and coactivator binding. The F-domain of ERα is important for coactivator selectivity and needs to be included in the structural studies proposed in aim 1.

ad Aim 2b:

- Analysis of a novel protein labeling system that involves protein splicing using the progesterone receptor (PR) as a test system: Cloning, expression, protein purification and protein splicing of a human PRαLBD-F/Intein chimera.

Although the solubility of the PRαLBD-F/Intein chimera and the yield of peptide coupling still needs to be increased, intein-catalyzed protein splicing appears to be the most promising experimental strategy to introduce a fluorophore into precise positions in the F-domain or H12.

Aim 1. Monitor the location and dynamic of the ER H12 in the absence and presence of various estrogens and develop a high throughput screen for the identification of new potential SERMs

Our results in aim 2 indicate that the structural analysis of ERβ is likely to be easier than that of ERα (higher solubility, no contribution of the F-domain to ligand- and coactivator-binding). Hence, in order to develop the fluorescence-based structural analysis system, thus far we have mainly focused on ERβ.

ad Aim 1a:

- Selection of sites for the introduction of fluorescence donors and acceptors based on available structural information: Potential donor sites in H12 - N496, A497, H498; potential acceptor sites - L477 (Agonist monitor); T323 (Antagonist monitor).
- Replacement of posssible donor sites by tryptophan using site-directed mutagenesis; expression of the mutated proteins in context of ERα±F-domain in *E. coli*, purification and estradiol binding studies: In the presence of the F-domain, the mutants N496W, A497W and H498W bind estradiol with similar affinity than wild type ER. In the absence of the F-domain the affinity of N496W and A497W decreases by a factor two, whereas the affinity of H498 for estradiol remains unchanged.

Thus, H498 seems to be the best candidate residue for the introduction of the fluorescence donor in H12.

ad Aim 1b:

- Comparison of overall yield and hormone binding activity of ERβ LBD expressed *in vitro* using bacterial, wheat germ or rabbit reticulocyte lysates: The overall protein yield was highest in the bacterial expression system, however the yield of hormone-binding competent receptor is significantly higher in reticulocyte lysate.

Unless we find an efficient strategy to separate apo- and holo-receptors, the rabbit reticulocyte systems appears to be the most effective ER *in vitro* translation system.

ad Aim 1c-e:
Surveying the structural features required for the efficient loading of t-RNAs with amino acids and for the recognition of artificially loaded t-RNAs by the ribosome. Construction of an ERβ LBD-H12-intein fusion protein.

Reportable Outcomes

Abstracts/Presentation:
C. Pullen, J. Goodley, B. Darimont
Novel strategies for the identification and characterization of selective estrogen receptor modulators
Annual Retreat of the Institute of Molecular Biology, University of Oregon, Silverfalls, Oregon, September 22/23, 2002
Era of Hope, Department of Defense Breast Cancer Research Program Meeting, Orange County Convention Center, Orlando, Florida, September 25-28, 2002

Employment:
Christian Pullen is currently interviewing for a scientist position at the drug development departments of Boehringer Ingelheim and Pfizer in Germany

Experience/Training:
Undergraduate research experience: Corinna Beyer
Research Associate research experience: Galina Kouzmitscheva, Lawrence Getubig
Postdoctoral training: Christian Pullen
Doctoral training: Joshua Goodley

Conclusions

While the characterization of ligand-bound ER LBDs by X-ray crystallography gave many interesting insights into the mechanisms of ligand binding and ligand interpretation, these structures do not provide a coherent explanation for the tissue- and receptor isotype-specific activity displayed by many ligands. There is increasing evidence that other receptor domains, such as the F-domain modulate these activities. Moreover, receptors bound to ligands such as SERMs, which display both agonistic and antagonistic activities, might differ from receptors bound to pure agonists or antagonists in terms of the dynamics rather than the nature of the ligand-induced structural changes. Our research provides one of the first attempts to monitor the dynamics of structural changes in the ER LBD upon ligand binding. These studies will not only give novel insights into the actions of SERMs but also provide a powerful strategy for the identification of novel SERMs that might improve the treatment of breast cancer.

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Appendices

Change in personnel:
Lawrence Getubig replaces Dr. Galina Kouzmitscheva who left the laboratory in September 2001.
Dr. Christian Pullen, who is presently interviewing for a position in the pharmaceutical industry in Germany, will be replaced by Joshua Goodley, a graduate student. Joshua joined the lab in June 2002 and is currently supported by an NIH training grant.

Biographical sketches for L. Getubig and J. Goodley are enclosed.

BIOGRAPHICAL SKETCHES

NAME	POSITION TITLE
Lawrence J. Getubig	Technician

EDUCATION/TRAINING

Institution and Location	Degree *(if applicable)*	Year(s)	Field of Study
University of Oregon	B.S.	1993-2001	Molecular Biology

RESEARCH EXPERIENCE

A. Positions:

1997 - 1999 Research Assistant, Laboartory of Dr. K. Sprague, University of Oregon
2001 - present Research Assistant, Laboratory of Dr. B. Darimont, University of Oregon

B. Honors:

Graduation in Molecular Biology (2001)

NAME	POSITION TITLE
Joshua Goodley	Graduate Student

EDUCATION/TRAINING

Institution and Location	Degree *(if applicable)*	Year(s)	Field of Study
University of California Santa Cruz	B.S. 2001	1997-2001	Biochemistry and Molecular biology
University of Oregon		2001-present	Biochemistry and Molecular Biology

RESEARCH EXPERIENCE

A. Positions:

Employer	Position/Title	Dates
University of California Santa Cruz	Undergraduate Student (Dr R Bogomolni)	98-00
University of Oregon	Graduate Student (Dr B. Darimont)	01-current

B. Honors:

SACNAS Undergraduate Research Grant summer 2001
Biophysical Society Undergraduate Researcher Travel Award 2001
University of Oregon Graduate Teaching Fellowship, 09/01 - 07/02;
National Institute of Health Molecular Biology Training Grant, since 08/02

NOVEL STRATEGIES FOR THE IDENTIFICATION AND CHARACTERIZATION OF SELECTIVE ESTROGEN RECEPTOR MODULATORS

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Estrogens promote the growth of particular tissues, and are involved in the cause of breast and endometrial cancers. Antiestrogens block the activity of estrogens and play important roles in the treatment of these cancers. However, they have many unwanted side effects such as increased risk for osteoporosis and heart diseases. The recently discovered selective estrogen receptor modulators (SERMs) provide a powerful alternative to antiestrogens. SERMs display profound cell-specific activity profiles blocking the activity of estrogen in breast, uterine or overian but not in other tissues. The goal of our work is to identify the mechanisms that control the activity of SERMs and to develop efficient high-throughput strategies for their identification.

Like natural estrogens, SERMs exert their activities by binding to the two estrogen receptors, ER alpha and ER beta, which are ligand-regulated transcription factors. Upon ligand binding, the position of alpha helix 12 (H12) in the ligand-binding domain of ER alpha and ER beta changes in a ligand-specific manner and regulates the interaction of these receptors with cellular corepressors and coactivators. The current hypothesis is that the cell-specific activity of SERMs is caused by cellular differences in the repertoir of coactivators and corepressors that recognize particular, SERM-induced receptor conformations.

During the past year our work has been focused on the identification of receptor-specific structural elements involved in the ligand-dependent repositioning of H12 and in the recruitment of corepressors and coactivators. We identified that the F-domain, a receptor-specific domain that follows H12, differentially enables ER alpha and ER beta to distinguish between different coactivators. Thus, this domain is most likely involved in the specific response of ER alpha and ER beta to particular SERMs. Additionally, we developed experimental strategies to introduce fluorescent labels into H12 or the F-domain. These fluorescent-labeled receptors will allow us to monitor the dynamics and structural reorganization of ER alpha and ER beta upon SERM binding and constitute the first step in the development of a high-throughput screen to identify novel SERMs.

The U.S. Army Medical Research Materiel Command under DAMD17-01-1-0359 supported this work.